



# Hyperthermophilic Archaea

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## Abstract

Archaea is considered to have the slowest evolution among all organisms. Studies on the genome structure of archaea indicate that it is similar in size to that of bacteria. However, most protein produced in archaea have eukaryotic features, which indicates a close relationship between archaea and eukarya in the course of evolution. The archaea and eukarya likely had a common ancestor not shared by the bacteria, and the first organisms to have diverged from the eukarya/archaea lineage were hyperthermophiles. Hyperthermophilic organisms therefore appear more closely related than all other organisms to the ancestor of all extant life, having evolved when the earth was much hotter than it is at present. Hyperthermophilic archaea can be divided into three groups by sulfur dependency; sulfur dependent, sulphate reducing genus and thermophilic methanogens. From Genome structure of hyperthermophilic archaea revealed that the small size genomes of hyperthermophiles may define the lower limit for their genetic capacity. Chromosomes of hyperthermophiles appear to be densely packed with genes, most of which are required for essential functions. This suggests that the earliest life forms may have had small genomes. Furthermore, the importance of archaea genomics is implicit in a universal phylogenetic tree; certain complex eukaryotic functions can be effectively studied in simpler archaeal systems, molecular structures can be inferred from thermostable archaeal proteins, and the functional essence of an enzyme or system can be revealed by a broader comparative analysis.

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Therefore, analysis of the archaea genome structure will provide us with a useful insight. The entire archaeal genome sequence of archaea would be useful to understand about the archaea themselves, thermophile, their relationship to the eukaryotic cell, the origin of the three primary lines of descent, and the nature of the most recent universal ancestor.

**Keywords :** archaea, hyperthermophile

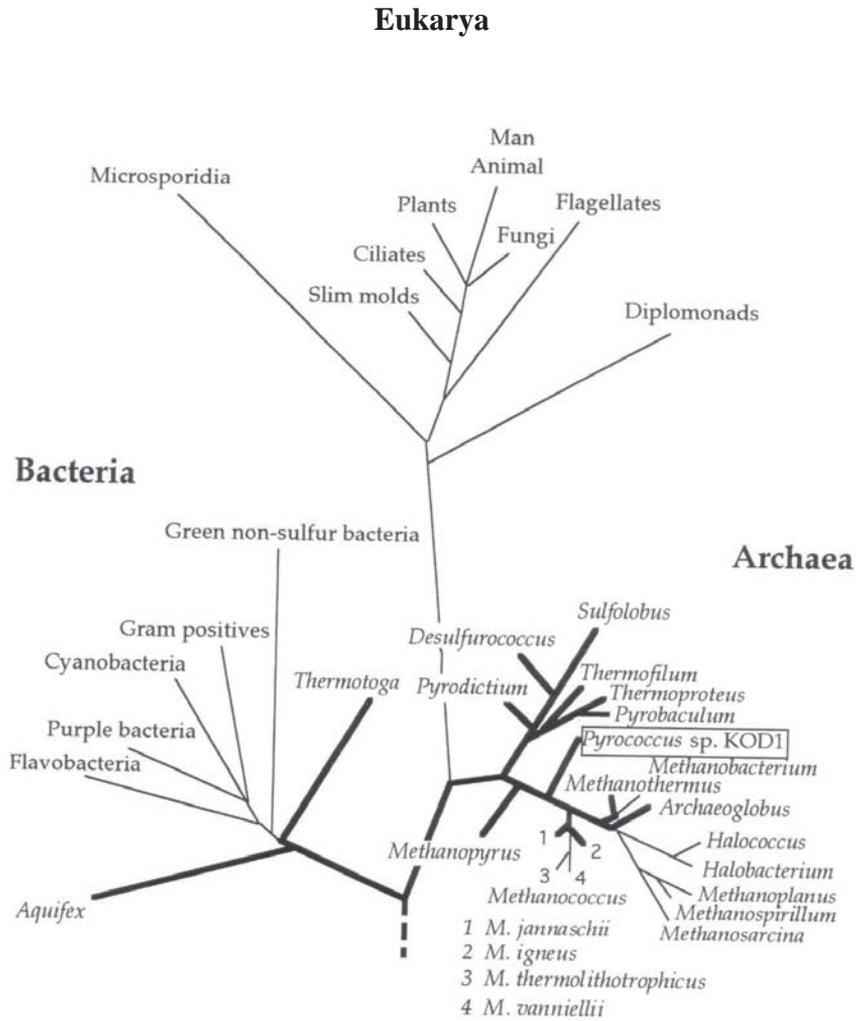
A phylogenic tree based on rRNA or protein sequences shows that all organisms are related, which suggests a common ancestor (Woese and Fox, 1977; Woese et al., 1990). However, organisms are quite unique and it is now known that all organisms are divided into three domains : eukarya, bacteria, and archaea (Fig 1). The archaeal domains comprises (at least) two major kingdoms, the Euryarcheota (Extreme Halophiles-Methanogens branch) and the Crenarchaeota (Sulfolobus-Thermoproteales branch). Interestingly, archaea is considered to have evolved the slowest among other domains. Some of the characteristics of archaeal organism are monocellular unit with single circular genome, lack of nuclei, no mitochondria or microsomes. Studies on the genome structure of archaea indicate that it is similar in size to that of bacteria. However, most protein produced in archaea have eukaryotic features, which indicates a close relationship between archaea and eukarya in the course of evolution (Gogarten et al. 1989; Iwabe et al. 1989; Trent et al. 1991; Rivera and Lake 1992). The archaea and eukarya likely had a common ancestor not shared by the bacteria, and the first organisms to have diverged from the eukarya/archaea lineage were hyperthermophiles (Woese et al. 1990; Wheelis et al. 1992). Hyperthermophilic organisms therefore appear more closely related than all other organisms to the ancestor of all extant life, having evolved when the earth was much hotter than it is at present (Woese 1987; Woese et al. 1990) Indeed, phylogenetic analysis suggests that the rest biology results from evolutionary pressures to adapt to temperatures lower than 100°C.

## Hyperthermophilic Archaea

Microorganisms that can grow at and above 100°C were discovered a decade ago, and about 20 different genera are now known. These so-called hyperthermophiles are the most ancient of all extant life; all but two genera (*Thermotoga* and *Aquifex*) are classified as *Archaea* (Woese et al. 1978; Woese 1987; Woese et al. 1990; Stetter et al. 1990; Adams 1993).

### Physiological features of hyperthermophilic archaea

Hyperthermophilic archaea can be divided into three groups by sulfur dependency (Adams 1993). As shown in Table 1, the majority falls into “sulfur dependent” category. They obtain energy for cell growth primarily by the metabolism of elemental sulfur ( $S^0$ ). The second group contains a unique sulfate reducing genus, *Archaeoglobus*, which is also hyperthermophilic. The third category consists of thermophilic methanogens, of which there are three genera, *Methanococcus*, *Methanothermus* and *Methanopyrus*, that can be classified as hyperthermophiles. It should be noted that several other methanogens are known that grow at temperatures up to 60°C or so. Thus, the majority of the thermophilic archaea are sulfur-dependent organisms.



**Fig. 1** Universal phylogenetic tree based on 16S rRNA sequences. Modified from Stetter (1996). Bold lines indicate the hyperthermophiles.

### **Sulfur-dependent archaea**

The sulfur-dependent archaea can be further subdivided into two main groups : anaerobic sulfur reducers, which grow at and above 90°C, and aerobic sulfur oxidizers, which are generally less thermophilic.

#### ***Anaerobic sulfur reducers***

The first category of sulfur dependent archaea includes the Thermoproteales, Thermococcales, and several as yet unclassified organisms. These are strictly anaerobic heterotrophs that are obligatory dependent on the reduction of sulfur to H<sub>2</sub>S for optimal growth. In fact, of these organisms only species of *Pyrococcus*, *Thermococcus* and *Hyperthermus* show growth in the absence of sulfur. Most of these anaerobic heterotrophs utilize only complex peptide mixtures such as yeast and meat extracts as carbon and nitrogen sources. Only a few of these organisms metabolize carbohydrates, including starch, glycogen, and maltose, but they also require peptides as a nitrogen source. Member of the group, Thermococcales, could be a good source for obtaining various polymer degrading enzymes. Some of these sulfur-dependent heterotrophs are able to grow autotrophically, using H<sub>2</sub> as the electron donor for sulfur reduction. All of these organisms are able to grow at 90°C and above. Most are of marine origin and several have been isolated near deep-sea vents. Only members of the Thermoproteaceae have been found in continental hot springs.

#### ***Aerobic sulfur oxidizers***

The second category of sulfur dependent archaea includes species of the Sulfolobales. In contrast to the sulfur-reducing heterotrophs, these are typically acidophilic aerobes that obtain energy for growth by the oxidation of sulfur to sulfuric acid. The Sulfolobales are generally less thermophilic than Thermoproteales and Thermococcales, with only species of *Acidianus* being able to grow at or above 90°C. In addition, they mainly inhabit continental sulfur-rich springs, although some species are also found near shallow marine volcanic vents. Remarkably, species of *Acidianus*

and *Desulfurolobus* also grow under anaerobic conditions by the reduction of sulfur ( $^{\circ}\text{S}$ ) to  $\text{H}_2\text{S}$  using  $\text{H}_2$  as the electron donor. *Stygiolobus* is unique among the Sulfolobales as it does not grow under aerobic condition. The unique genus *Thermoplasma*, which is situated at an isolated position in the phylogeny, also belongs to this category. Species of *Thermoplasma* are facultatively anaerobic heterotrophs. They grow optimally near  $60^{\circ}\text{C}$  both with and without sulfur ( $^{\circ}\text{S}$ ) and can utilize monosaccharides as a carbon source.

In considering the effects of temperature on growth physiology, it is apparent that at the present upper temperature limits of life, the predominant metabolism is strictly anaerobic, heterotrophic sulfur reduction. In addition, almost of the hyperthermophilic species require complex organic mixtures as carbon and nitrogen sources, although a few species (including the methanogens) are able to grow autotrophically with  $\text{H}_2$  as the electron donor. The ability to use  $\text{O}_2$  as a terminal electron acceptor is very limited at temperature above  $90^{\circ}\text{C}$  due to low solubility of oxygen (Stetter et al. 1990). Since both of these gases are often present in volcanic exhalations (Williams and McBirney, 1979), utilization of sulfur instead of oxygen appears to be the predominant way of energy conversion by hyperthermophiles (Adam 1993). In contrast, at slightly lower temperatures, it is the aerobic, sulfur oxidizing autotrophs that predominate.

**Table 1** Classification of thermophilic and hyperthermophilic archaea

Order (Family) Genus	$T_{\max}^a$	physiology <sup>b</sup>	Donors <sup>c</sup>	Acceptors <sup>c</sup>	Habitat
<b>Sulfur-dependent archaea<sup>e</sup></b>					
Thermoproteales (Thermoproteaceae)					
<i>Pyrobaculum</i>	102°	hetero (auto)	org N (H <sub>2</sub> )	S°	c
<i>Thermofilum</i>	100°	hetero	org N	S°	c
<i>Thermoproteus</i>	92°	hetero (auto)	org C,N (H <sub>2</sub> )	S°	c
<b>Thermoproteales (Desulfurococcaceae)</b>					
<i>Pyrodictium</i>	110°	hetero (auto)	org C,N (H <sub>2</sub> )	S°	m/d
<i>Staphylothermus</i>	98°	hetero	org N	S°	m/d
<i>Thermodiscus</i>	98°	hetero	org N	S°	m
<i>Desulfurococcus</i>	90°	hetero	org N	S°	m/d
<b>Thermococcales</b>					
<i>Pyrococcus</i>	105°	hetero	org C,N	S° (-) <sup>f</sup>	m
<i>Thermococcus</i>	97°	hetero	org C,N	S° (-)	m/d
<b>(Unclassified)</b>					
<i>Hyperthermus</i>	110°	hetero	org N (H <sub>2</sub> )	S° (-)	m
“ES-4”	108°	hetero	org C,N	S°	d
“GB-D”	103°	hetero	org N	S°	d
“GE-5”	102°	hetero	org N	S°	d
“ES-1”	91°	hetero	org C,N	S°	d
<b>Sulfolobales</b>					
<i>Acidianus</i>	96°	auto	S°, H <sub>2</sub>	O <sub>2</sub> (S°)	m/c
<i>Sulfolobus</i>	87°	auto	S°, H <sub>2</sub> (org C, N)	O <sub>2</sub> (S°)	c
<i>Desulfurolobus</i>	87°	auto	S°, H <sub>2</sub>	O <sub>2</sub> (S°)	m/c
<i>Stygiolobus</i>	88°	auto	H <sub>2</sub>	S°	c
<i>Metallosphaera</i>	80°	auto	S° (org N)	O <sub>2</sub>	c
<b>Thermoplasmatales</b>					
<i>Thermoplasma</i>	67°	hetero	org C,N	(-), O <sub>2</sub> , S°	c
<b>SULFATE-REDUCTING ARCHAEA</b>					
<i>Archaeoglobus</i>	95°	hetero (auto)	org C (H <sub>2</sub> )	SO <sub>4</sub> , S <sub>2</sub> O <sub>3</sub>	m/d
<b>METHANOGENIC ARCHAEA</b>					
<i>Methanococcus</i>	91°	auto	H <sub>2</sub>	CO <sub>2</sub>	m/d
<i>Methanothermus</i>	97°	auto	H <sub>2</sub>	CO <sub>2</sub>	c
<i>Methanopyrus</i>	110°	auto	H <sub>2</sub>	CO <sub>2</sub>	m/d

- <sup>a</sup> Minimum growth temperature.
- <sup>b</sup> Indicated whether species of a genus are heterotrophs (hetero) or autotrophs (auto) or both.
- <sup>c</sup> Electron donors and acceptors.
- <sup>d</sup> Isolated from continental (c), shallow marine (m), or deep-sea (d) geothermal areas.
- <sup>e</sup> The sulfur-dependent genera are grouped in separate order, except for *Hyperthermus*, ES-1, ES-4, GB-D, and GE-5, which have yet to be classified.
- <sup>f</sup> (-) indicates growth in the absence of S<sup>0</sup>. (Modified from Adams 1993.)

### Genome structure of hyperthermophilic archaea

Limited information is available on chromosome organization of archaea. Previously, circular chromosomal maps of *Thermococcus celer* (1890 kb) (Noll 1989), *Methanococcus voltae* (1880 kb) (Sitzmann and Klein 1991), *Haloferax volcanii* DS2 (2920 kb) (Charlebois 1991), *Haloferax mediterranei* (2900 kb) (López-García et al. 1992), and *Sulfolobus acidocaldarius* 7 (2760 kb) (Kondo et al. 1993) were reported. The genome size of thermophiles are comparable to those of mesophiles. Generally, hyperthermophiles have a smaller size genome than that of the well studied mesophiles, such as *Escherichia coli* (4672 kb) (Smith et al. 1987, Kroger et al. 1993), *Bacillus subtilis* (4188 kb) (Itaya and Tanaka 1991), *Streptomyces coelicolor* (8 Mb) (Kieser et al. 1992), and *Myxococcus xanthus* (9 Mb) (Chen et al. 1990). While hyperthermophilic bacteria, *Thermus thermophilus* (Borges and Gergquist 1993), *Thermotoga maritime* (Kim et al. 1993), and *Aquifex pyrophilus* (Shao et al. 1994) possess 1740 kb, and 1620 kb genomes, respectively. Among the hyperthermophilic archaea whose genome size are known, *Methanococcus jannaschii* (1660 kb) (Bult et al. 1996) has the smallest genome. The small size genomes of hyperthermophiles may define the lower limit for their genetic capacity. Chromosomes of hyperthermophiles appear to be densely packed with genes, most of which are required for essential functions. This suggests that the earliest life forms may have had small genomes.

### Genomic analysis of hyperthermophilic archaea

The importance of archaea genomics is implicit in a universal phylogenetic tree; certain complex eukaryotic functions can be effectively studied in simpler archaeal systems, molecular structures can be inferred from thermostable archaeal proteins, and the functional essence of an enzyme or system can be revealed by a broader comparative analysis. Therefore, analysis of the archaea genome structure will provide us with a useful insight.

In General, gene regulation systems of archaea are considered to be similar to those of eukarya. Of the three main multicomponent information processing systems (replication, transcription, and translation), the enzymes which comprise the translation machinery are most commonly compared. Elongation factors exhibit the most similarities to their eukaryotic counterpart (Iwabe et al. 1989). Aminoacyl-tRNA synthetases from archaea resemble eukaryotic aminoacyl-tRNA synthetases more closely than the bacterial form (Brown et al. 1995, Imanaka et al. 1995) RNA polymerase from archaea also share common features from their eukaryotic counterparts (Zillig et al. 1993, Thomm 1996). Bacterial RNA polymerase is basically composed of  $\alpha$ ,  $\hat{\alpha}$ ,  $\hat{\alpha}'$  and  $\sigma$  subunits. However, archaeal RNA polymerase consists of at least 12 kinds of subunits, showing remarkable similarity to eukaryotic counterpart, especially PoIII. The archaeal transcription initiation system is almost identical to that found in eukaryotes, but is different from the bacterial system. The central molecules in the former systems are TATA-binding protein (TBP) and transcription factor B (TFIIB and TFIIB in eukaryotes, or simply TFB). In the eukaryotic systems, TBP and TFB are part of larger complexes, and additional factor (such as TFIIA and TFIIF) are used in the transcription process. In addition, TBP of hyperthermophile is abnormally acidic, suggesting unknown positively charged protein is also involved in the transcription initiation event (Rashid et al. 1995).

In contrast, archaeal genes which encode proteins involved in carbon/nitrogen metabolism and membrane transport machinery are homologues of bacterial genes. Based on the genome analysis of hyperthermophilic methanogenic archaeon *M. jannaschii*, the genome includes many representatives of the bacterial ABC transporter family (Bult et al. 1996). More than 20 predicted protein-coding regions have sequence similarity to polysaccharide biosynthesis enzymes. These genes have only bacterial homologues or are most closely related to their bacterial counterparts.

Recently, the first entire archaeal genome sequence, *Methanococcus jannaschii*, has been determined (Bult et al. 1996). Other genome projects involving *Pyrococcus furiosus* and *Sulfolobus acidocaldarius* are in progress. These information would be useful to understand about the archaea themselves, thermophile, their relationship to the eukaryotic cell, the origin of the three primary lines of descent, and the nature of the most recent universal ancestor.

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